

RESEARCH PAPER

Assessment of genetic variability in landraces of rice (*Oryza Sativa* L) for grain iron and zinc content using energy-dispersive X-Ray fluorescence spectrophotometer (EDXRF)

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Abstract: Rice (*Oryza sativa* L.) is a staple food for over half of the global population and plays a crucial role in ensuring food and nutritional security. However, many rice-dependent populations suffer from widespread deficiencies of essential micronutrients, particularly iron (Fe) and zinc (Zn), leading to malnutrition and hidden hunger. Biofortification provides a sustainable solution to improve the nutritional value of rice, which requires the identification and assessment of diverse germplasm to uncover genetic variation in Fe and Zn concentrations. Present study evaluated grain Fe and Zn content in 100 traditional rice landraces using energy-dispersive X-ray fluorescence (EDXRF) spectrometry during the *kharif* seasons of 2023-24. The Fe concentrations ranged from 5.7 to 14.1 ppm, while Zn concentrations varied from 16.7 to 42.5 ppm, revealing significant genetic variation among the landraces. The landrace Bangar Gundu showed the highest Zn concentration (38.25 ppm) and moderate Fe content (14 ppm), while five other landraces—Choman, Adnen Kelthe, Haldoddiga, Karibhatta, and Uma—exhibited Zn levels exceeding 29 ppm. Seasonal variability in asymmetry and tail behaviour of iron and zinc concentration among rice landraces was obtained through the distribution analysis. Zinc was particularly evident with pronounced right-skewness and leptokurtic distribution during *kharif* 2024, suggesting the existence of high-value extreme genotypes. These results demonstrate the potential of traditional landraces as valuable genetic resources for biofortification programs targeting nutrient-dense rice varieties.

Key words: Biofortification, Energy-dispersive X-ray fluorescence (EDXRF), Hidden hunger, Iron, Micronutrient deficiencies, Rice, Traditional landraces, Zinc

Introduction

Rice (*Oryza sativa* L.), an ancient agricultural crop, remains one of the world's main food sources. It is a rich source of complex carbohydrates, providing 50–80% of daily calories, along with essential proteins, minerals and vitamins (Calayugan *et al.*, 2020). Rice is the primary staple for more than 2 billion people in Asia and hundreds of millions in Africa and Latin America. Asia is often called the “Rice Basket of the World” because it produces and consumes over 90% of the global rice supply, highlighting the region's high population density and dependence on rice. In 2023, global rice production reached 799.99 million tons, cultivated on 168.35 million hectares (FAOSTAT 2023). In India, rice production was 137.82 million tons, grown on 47.82 million hectares (Indiastat, 2023-24). These figures underline rice's vital role in global food security and its importance in sustaining the livelihoods and nutrition of billions.

Globally, an estimated more than two billion people suffer from micronutrient deficiencies or malnutrition due to iron and zinc. This form of malnutrition is also called hidden hunger. 2.5 billion adults are overweight or obese, while 390 million are underweight. It is estimated that 149 million children under 5 are stunted (too short for their age), 45 million are wasted (too thin for their height), and 37 million are overweight or obese (WHO 2022). Iron (Fe) and zinc (Zn) are essential micronutrients vital for physiological functions, including tissue growth, immune response and enzymatic activity. Iron is crucial for

haemoglobin synthesis and oxidative metabolism, while zinc supports the biosynthesis and metabolism of macronutrients and nucleic acids. Deficiencies in Fe and Zn can cause anemia, impaired development, weakened immunity and stunted growth. To address these issues, the Food Safety and Standards Authority of India (FSSAI) recommends dietary allowances of Fe (17, 21 and 35 mg/day) and Zn (12, 10, and 12 mg/day) for men, women and pregnant women, respectively.

Rice, as a staple food for much of the global population, serves as an ideal vehicle for delivering essential micronutrients like iron (Fe) and zinc (Zn). Enhancing both the concentration and bioavailability of these micronutrients in rice grains is crucial to addressing deficiency-related disorders in rice-dependent populations. Biofortification has emerged as a sustainable and cost-effective strategy for developing micronutrient-enriched cereal crops. A prerequisite for such programs is the screening of diverse germplasm to identify genetic variation for the target traits. Screening of rice genotypes for grain zinc content is, therefore, the first step in the plant breeding program for the development of high-yielding rice varieties along with high grain zinc content (Chandu *et al.*, 2020). Assessment of genetic variability is fundamental in rice breeding programs for selection, conservation of various rice landraces and proper utilization (Raza *et al.*, 2020).

Traditional rice landraces, known for their genetic diversity and valuable nutritional traits, provide a vital resource for breeding programs aimed at developing nutrient-dense rice varieties. Landraces are important genetic material that possesses potential characteristics for crop improvement and development programs (Christina *et al.*, 2021) are essential.

The present study aimed to evaluate rice genotypes for iron (Fe) and zinc (Zn) concentrations in brown rice using the energy-dispersive X-ray fluorescence (EDXRF) technique. Anuradha *et al.* (2012) screened 126 rice lines in XRF and showed that wild rice accessions have higher grain Fe and Zn concentration. Maganti *et al.* (2020) studied the variation of grain Fe and Zn concentration in 159 rice germplasm using XRF. They observed a positive correlation between the two micronutrients. The primary goal was to identify genotypes with elevated Fe and Zn levels, which could serve as potential candidates for biofortification programs to enhance the nutritional value of rice and address micronutrient deficiencies.

Material and methods

Experimental site

The experiment was conducted at AICRP (Voluntary Centre), ARS (Paddy), Sirsi, affiliated to the University of Agricultural Sciences, Dharwad.

Plant materials

A set of hundred local landraces collected from Karnataka was evaluated during two seasons, *kharij* 2023 and *kharij* 2024 using a Randomized Complete Block Design with two replications. Healthy crop conditions were maintained by following the recommended package of practices.

Estimation of iron and zinc content in grains

The concentrations of iron (Fe) and zinc (Zn) in brown rice samples were estimated using a non-destructive energy-dispersive X-ray fluorescence spectrometry (EDXRF) (Rao *et al.*, 2014) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK) at the Indian Institute of Rice Research (IIRR), Hyderabad. For each genotype, 15 g of dried paddy was de-husked using a non-metallic de-husker. Following de-husking, the brown rice samples were cleaned to remove broken grains by sieving. The dust and debris were cleaned by rubbing them against tissue paper 4 times for 1 min. Five g of each cleaned sample was weighed and placed in sample cup for analysis. The cups were gently shaken to ensure uniform sample distribution before measurement. The concentrations of Fe and Zn were determined and expressed in parts per million (ppm) of grain weight.

Statistical analysis

All statistical analyses were done using Microsoft EXCEL.

Results and discussion

Iron and zinc content in the brown rice grains

The descriptive statistics of 100 local landraces for grain iron and zinc content in two seasons and mean of two seasons were analyzed and results reveal that, the iron concentration ranged from 5.7 ppm to 13.9 ppm in season 1 and 6.4 ppm to 14.1 ppm in season 2, and the zinc concentration ranged from 16.7

ppm to 34 ppm in season 1 and 19.3 to 42.5 in season 2 (Table 1) and the mean grain iron concentration for two seasons ranged from 6.55 ppm to 14 ppm and most of the genotypes lay between 8 ppm to 12 ppm (84) in brown rice. The mean grain zinc concentration for two seasons ranged from 18.95 ppm to 38.25 ppm, and most of the genotypes lay between 25-30 ppm (93) (Table 1, Fig 1). The overall mean of 100 landraces for iron was 8.89 ppm and for zinc was 24.82 ppm (Table 2). Kaur *et al.* (2024) characterized 206 rice germplasm lines and recorded sufficient variability in the brown rice of for grain Fe (4.6-37.5, 6.7-16.1 ppm) and Zn (16.3-41.0, 15.0-58.2 ppm) contents. Maganti *et al.* (2020) observed that Iron concentration varied from 6.9 to 22.3 mg/kg, whereas zinc concentration ranged from 14.5 to 35.3 mg/kg in unpolished, brown rice. Similar observations were reported by Parik *et al.* (2019) in their evaluation of 192 diverse rice germplasm lines for iron and zinc content in brown rice which ranged between 6.3 µg/g -24.5 µg/g and 15.4 µg/g -39.40 µg/g, respectively. Another study reported iron concentration ranging from 6.6 µg/g to 16.7 µg/g and zinc concentration from 7.1 µg/g to 32.4 µg/g in a screening of 192 lines (Nachimuthu *et al.*, 2014).

Classification of the genotypes based on the micronutrient density

The 100 local landraces were classified based on the iron and zinc concentration in the dehusked brown rice into low, moderate and high. Brown rice genotypes with iron content less than 12 mg/kg were categorized as low (99 genotypes), iron content between 12.1 and 15 mg/kg was grouped under moderate (1 genotype) and more than 15.1 mg/kg (0 genotypes) were considered high (Fig 2a). Similarly for zinc, less than 20 mg/kg (2 genotypes) was considered low, between 20.1 and 29 mg/kg as moderate (92 genotypes) and more than 29 mg/kg (6 genotypes) was grouped as high (Fig 2b). A similar kind of classifying the genotypes into different groups was done by Maganti *et al.* (2020) she grouped 159 genotypes into different classes, 41,99,19 genotypes grouped into low, moderate and high for iron content in brown rice, similarly 18,126 and 15 genotypes were classified into low, moderate and high for zinc concentration in grains.

The lowest concentration of iron was observed in the landrace Yellapur Bhatta (SRS-G-83), at 6.55 ppm and the highest in Bangar Gundu (SRS-G-2), at 14 ppm. The lowest concentration of zinc was noted in Mathalgia (SRS-G-24) at 18.95 ppm and the highest was recorded in Bangar Gundu (SRS-G-2) at 38.25 ppm. The six genotypes Choman, Adnen Kelthe, Haldoddiga, Karibhatta, Uma and Bangar Gundu has shown the highest concentration of zinc >29ppm. Among these landraces, only Bangar Gundu has shown a moderate level of iron concentration with 14 ppm. Chandu *et al.* (2020) evaluated 100 germplasm for iron and zinc content in grains using ED-XRF and reported that iron concentration varied from 1.6 to 15.2 ppm whereas zinc concentration ranged from 6.2 to 33.2 ppm in brown rice samples and 10 genotypes N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M with high grain iron and zinc concentration. Sahu *et al.* (2017) studied on brown

Table 1. Mean grain iron and zinc concentrations for 100 landraces for 2 seasons.

	Name of the landraces	kharif 2023		kharif 2024		Mean Fe (ppm)	Mean Zn (ppm)
		Fe(ppm)	Zn(ppm)	Fe(ppm)	Zn(ppm)		
SRS-G-1	Haldoddiga	9.6	28.6	9.5	32.6	9.55	30.6
SRS-G-2	Bangar Gundu	13.9	34	14.1	42.5	14	38.25
SRS-G-3	Chittimutyallu	7.2	26.5	12.3	27.3	9.75	26.9
SRS-G-4	Gulwadi Sannakki	9.2	27.7	9	26.1	9.1	26.9
SRS-G-5	Adnenukelthi	8	22.4	9.6	24.2	8.8	23.3
SRS-G-6	Adnen Keltthe	9.4	29.1	10.1	30.7	9.75	29.9
SRS-G-7	Uma	10.5	32.4	9.6	35.8	10.05	34.1
SRS-G-8	Gudda Dani	8.5	23.9	11.3	31.4	9.9	27.65
SRS-G-9	Karibhatta	8.6	27.1	10.8	37.8	9.7	32.45
SRS-G-10	Chitga	10.2	25.3	10.5	28.7	10.35	27
SRS-G-11	Jadda Bhatta	7.8	25.7	10.9	31.2	9.35	28.45
SRS-G-12	Kaime	8.9	23.7	10.5	34.1	9.7	28.9
SRS-G-13	Halga	7	20.3	9.3	24	8.15	22.15
SRS-G-14	Choman	9.6	29.6	12.3	29	10.95	29.3
SRS-G-15	Komal	7	18.4	10.8	31.5	8.9	24.95
SRS-G-16	Case Bhatta	9.6	27	8.2	24	8.9	25.5
SRS-G-17	Hegge	8.2	19.9	10.6	29.8	9.4	24.85
SRS-G-18	Mysore Sanna	9.4	27.1	9.7	26.5	9.55	26.8
SRS-G-19	Table Rice	8.8	24.5	10.4	28.7	9.6	26.6
SRS-G-20	Bora (Assam)	9.1	21.2	8.9	24.1	9	22.65
SRS-G-21	Asha	9.3	22.8	9.8	25.1	9.55	23.95
SRS-G-22	Mysore Sanna 1	8.2	24.5	8.9	26	8.55	25.25
SRS-G-23	Sanna Mullare	7.3	24.2	9.5	21.9	8.4	23.05
SRS-G-24	Mathalgya	7.3	18.6	9.3	19.3	8.3	18.95
SRS-G-25	Mysore Mallige	10.7	24.7	11.1	24.2	10.9	24.45
SRS-G-26	Coimbatore	7.1	24.3	10.5	26.6	8.8	25.45
SRS-G-27	Selam Sanna	7.7	18.2	8.9	25.1	8.3	21.65
SRS-G-28	Masurolya	8.1	19	9.5	26.1	8.8	22.55
SRS-G-29	Siri	6.4	16.7	10.7	23	8.55	19.85
SRS-G-30	Madras Sanna	8.7	20.3	9.4	25.5	9.05	22.9
SRS-G-31	Kari Kagga	9.2	21.4	10.8	29.8	10	25.6
SRS-G-32	Biliakki	10.3	24.5	10.3	27.1	10.3	25.8
SRS-G-33	Padmarekha	9.6	23.5	7.9	19.8	8.75	21.65
SRS-G-34	Tunga	8	21.8	11.4	29.3	9.7	25.55
SRS-G-35	Edi Kuni	7.7	18.9	9.5	25	8.6	21.95
SRS-G-36	Gandhasale	10.1	22.3	10.1	20.4	10.1	21.35
SRS-G-37	Bilidadi Marthlygya	9.3	20.6	8.1	22.6	8.7	21.6
SRS-G-38	Dodda Halga	8.5	21.7	12.7	30.6	10.6	26.15
SRS-G-39	Mahsuri	8.8	25.5	8.3	23.4	8.55	24.45
SRS-G-40	Padma Rekha	7.7	22.1	7.8	21.4	7.75	21.75
SRS-G-41	Ambemori	9.3	22.8	10.1	27	9.7	24.9
SRS-G-42	Rajkamal	9.3	26.4	8.6	25.2	8.95	25.8
SRS-G-43	Karna	7.8	22.8	10.6	25.2	9.2	24
SRS-G-44	Madras Sanna 1	9.5	21.4	10.8	25.4	10.15	23.4
SRS-G-45	Parimala Sanna	6.8	18.8	10.6	24.5	8.7	21.65
SRS-G-46	Farmolya	8.1	24.3	9.2	26	8.65	25.15
SRS-G-47	Shakti	8.4	21.9	9	24.3	8.7	23.1
SRS-G-48	Zigoratgya	8	21.4	8.7	23.9	8.35	22.65
SRS-G-49	Bantwal	8.8	23.2	8	23.9	8.4	23.55
SRS-G-50	Kumkum Kesari	9.7	23	10.4	20.8	10.05	21.9
SRS-G-51	Karthika	8.3	20.1	11.2	24.8	9.75	22.45
SRS-G-52	Bili Mullare	8	18.1	8.7	24.2	8.35	21.15
SRS-G-53	Ratnachuda	9.1	24.2	9.4	25.9	9.25	25.05
SRS-G-54	Mysure Sanna	8	24.5	8.2	24.5	8.1	24.5
SRS-G-55	Uma 1	8.5	23.3	7.6	22.4	8.05	22.85
SRS-G-56	Dodda Bhatta	8.9	24	7.5	24.1	8.2	24.05
SRS-G-57	Medhini Sannabhatta	8.8	27.4	9.7	22.1	9.25	24.75
SRS-G-58	Coimbatore Sanna	6.7	22.9	7.5	26.1	7.1	24.5
SRS-G-59	Padma Recha	8.3	19	7.8	23.5	8.05	21.25

SRS-G-60	Geerge Sanna	8.8	28.7	11.1	19.8	9.95	24.25
SRS-G-61	Somasele	10	17.5	6.4	22.5	8.2	20
SRS-G-62	Intan Cross	6.6	18.5	8.2	23.3	7.4	20.9
SRS-G-63	Raj Kaima	8.4	23.6	10.3	28	9.35	25.8
SRS-G-64	Diamond Sona	8.4	24.1	9.8	25.1	9.1	24.6
SRS-G-65	Gujagunda	9	26	7.2	24.8	8.1	25.4
SRS-G-66	Bili Intan	10.3	25	7.8	25.2	9.05	25.1
SRS-G-67	Migola	9.2	21.4	8.9	26.9	9.05	24.15
SRS-G-68	Marnomi Doddiga	7.8	18.7	7.9	26	7.85	22.35
SRS-G-69	Vanasurya	8.8	26.2	8.5	30	8.65	28.1
SRS-G-70	Dodda Bilibhatta	7.5	25	6.8	24	7.15	24.5
SRS-G-71	Gudda Bhatta	7	25.2	8.5	26.2	7.75	25.7
SRS-G-72	Dodda Mullare	8	21.8	8.7	26	8.35	23.9
SRS-G-73	Thogarsi	7	24.7	9	25.1	8	24.9
SRS-G-74	Madhura	7.1	26.1	7.8	21	7.45	23.55
SRS-G-75	Kabbaga	6	21.7	9.6	26.8	7.8	24.25
SRS-G-76	Byalearya	6.2	20.6	7.5	21.5	6.85	21.05
SRS-G-77	Gumgalhi	8.6	24.3	7.5	23.2	8.05	23.75
SRS-G-78	Bulbuli	7.3	22.6	7.3	21.8	7.3	22.2
SRS-G-79	Sanna Bili Batta	7.9	23	8.5	23	8.2	23
SRS-G-80	Ubarmunda	7.9	28	9	25.9	8.45	26.95
SRS-G-81	Mysore Sanna 2	8.7	27	8.5	24.1	8.6	25.55
SRS-G-82	Bara Ratnachudi	7.4	22.8	9.2	23.6	8.3	23.2
SRS-G-83	Yellapur Bhatta	5.7	28	7.4	22.9	6.55	25.45
SRS-G-84	Honasu	8.9	32.1	7.6	25.6	8.25	28.85
SRS-G-85	Sampedala	9.7	25.4	8.3	24.1	9	24.75
SRS-G-86	Marnomi Gudda Bhatta	9.4	23.5	9.2	27.4	9.3	25.45
SRS-G-87	Onedu Kaddi	9	24.8	8.1	25.1	8.55	24.95
SRS-G-88	Hegge 1	9.8	24	8.3	26.4	9.05	25.2
SRS-G-89	Kannur	9.8	28.4	10.3	26.7	10.05	27.55
SRS-G-90	Esadi	8.2	21.1	9.5	26.5	8.85	23.8
SRS-G-91	Bili Kane Hegge	10.7	29.5	8.9	26.4	9.8	27.95
SRS-G-92	Soratgya	8.3	24.7	8.3	28.8	8.3	26.75
SRS-G-93	Bilidadi Mathalga	9	27.3	9.8	23.5	9.4	25.4
SRS-G-94	Neer Mulga	9	21.2	9.2	25.6	9.1	23.4
SRS-G-95	Madras Sanna 2	6.7	20.7	10.2	25.7	8.45	23.2
SRS-G-96	Bili Intan 1	8.3	24.9	7	23	7.65	23.95
SRS-G-97	Mendoni	8.4	26.5	9.7	31.2	9.05	28.85
SRS-G-98	K.S.39	8.2	29.6	7.7	21	7.95	25.3
SRS-G-99	Jondhale	9.9	31.4	7.9	19.3	8.9	25.35
SRS-G-100	Ratnachuda 1	7.3	22.1	8.7	27.3	8	24.7

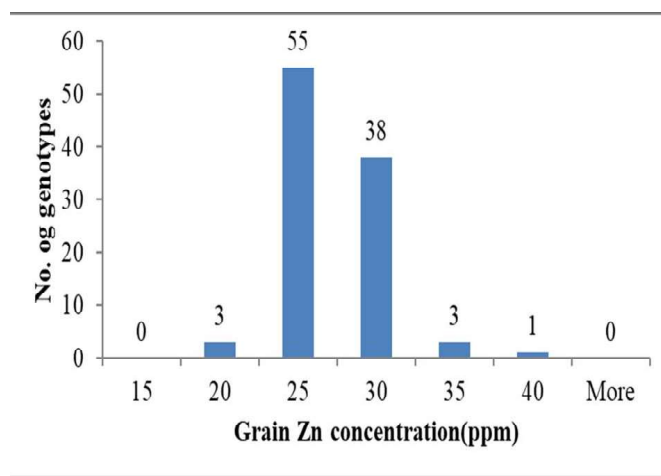
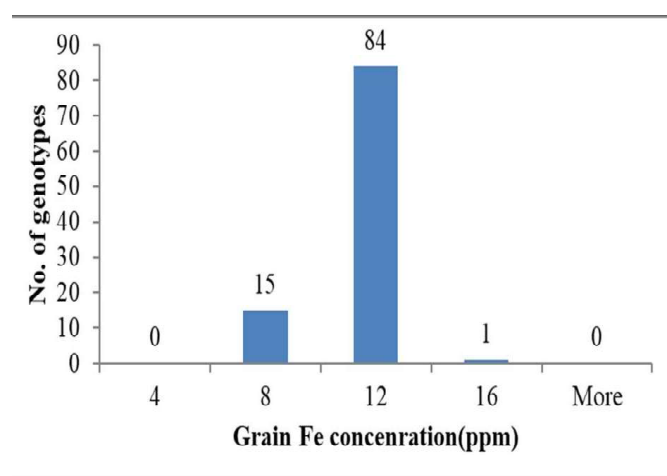


Fig 1. Frequency distribution of grain Iron and Zinc concentration in 100 rice landraces.

Table 2. Descriptive statistics of grain Fe and Zn in 100 rice landraces for 2 seasons

	Samples	Minimum	Maximum	Mean	Range	Variance	Standard Deviation	Standard Error	Skewness	Kurtosis
Fe (ppm)- <i>kharif</i> 2023	100	5.70	13.90	8.51	8.20	1.48	1.22	0.12	0.65	2.86
Zn (ppm)- <i>kharif</i> 2023	100	16.70	34.00	23.87	17.30	12.17	3.49	0.35	0.38	0.12
Fe (ppm)- <i>kharif</i> 2024	100	6.40	14.10	9.27	7.70	1.92	1.38	0.14	0.57	0.61
Zn (ppm)- <i>kharif</i> 2024	100	19.30	42.50	25.76	23.20	14.27	3.78	0.38	1.44	3.96
Mean Fe (ppm)	100	6.55	14.00	8.89	7.45	1.04	1.02	0.10	1.19	5.42
Mean Zn (ppm)	100	18.95	38.25	24.82	19.30	8.47	2.91	0.29	1.45	4.58

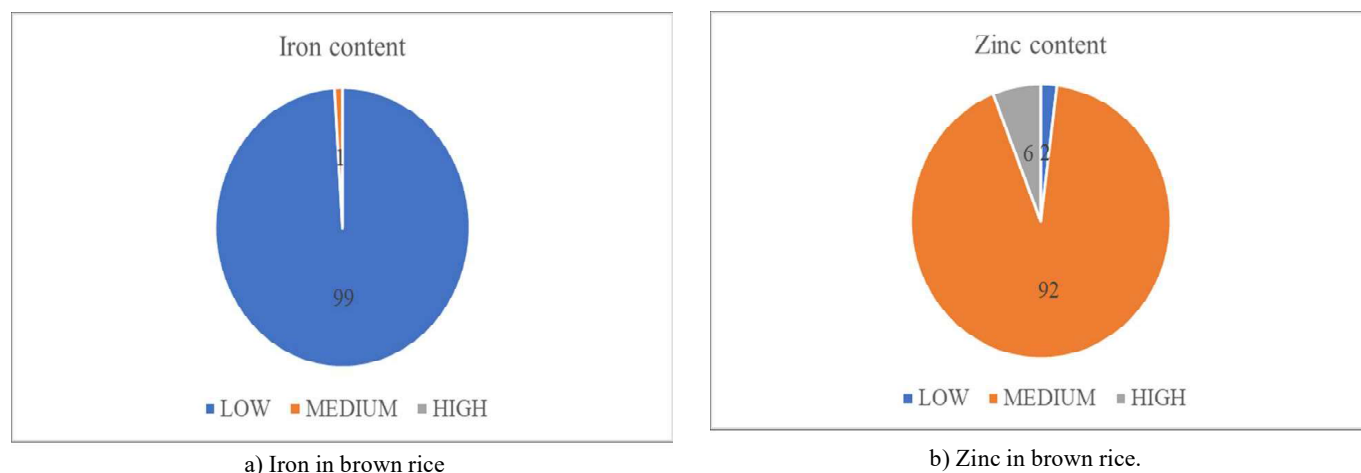


Fig 2. Classification of genotypes based on Iron and Zinc content.

rice of 47 germplasm and noted that the iron content ranged from 6.35 g/g to 17.90 µg/g, whereas zinc content from 13.50 µg/g to 30.21 µg/g in brown rice. The accessions, Peeleeluchai (135131) and Rajniti (A) had the highest grain iron and zinc content. Patil *et al.* (2015) screened the 60 rice accessions and found that iron concentration ranged from 3.38 ppm to 36.99 ppm and zinc from 3.32 ppm to 42.49 ppm. Among the genotypes ADT-43, HMT and Parimala sanna were found to be high for both iron and zinc (36.99ppm and 42.27ppm), (14.52ppm and 30.45ppm) and (12.90ppm and 27.43ppm), respectively. The results showed significant diversity among the landraces for their iron and zinc content in brown rice.

Skewness and kurtosis for iron and zinc

The distributional evaluation of grain micronutrient content among the rice landraces produced significant seasonal differences in skewness and kurtosis for both iron (Fe) and zinc (Zn) (Table 2). During *kharif* 2023, Fe content exhibited moderate right skewness (skewness = 0.65), indicating a concentration of landraces with lower Fe concentrations and a few with elevated values. The kurtosis of 2.86 was nearly that of a normal distribution, indicating an even frequency of extreme values. Zn concentration in the same season had mild right-skewness (skewness = 0.38), indicating almost symmetrical distribution with a slight bias towards higher values, whereas

its kurtosis value of 0.12 reflected a very platykurtic, flat distribution with few outliers. During *kharif* 2024, Fe content had a moderately right-skewed distribution (skewness = 0.57) and a flatter shape (kurtosis = 0.61), indicative of fewer extremes. Zn content, on the other hand, showed strong right-skewness (skewness = 1.44), indicating the presence of higher concentrations of smaller values with fewer extreme highs, and a leptokurtic distribution (kurtosis = 3.96), signifying a higher peak and fatter tails with a greater frequency of outliers. These patterns of distribution demonstrate the underlying micronutrient trait variation, which is essential in the identification and use of nutritionally better rice landraces.

Conclusion

This study revealed significant genotypic variation in grain iron and zinc concentrations among traditional rice landraces, highlighting their potential as a valuable resource for biofortification. In particular, six landraces—Choman, Adnen Kelthe, Haldoddiga, Karibhatta, Uma, and Bangar Gundu—demonstrated remarkably high zinc concentrations (>29 ppm), with Bangar Gundu also exhibiting a moderate iron level (14 ppm). These genotypes serve as promising donors for improving rice micronutrient content through breeding. Their use can help develop nutrient-rich varieties to combat hidden hunger and support the genetic diversity essential for sustainable nutritional security.

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